INVESTIGATION OF SOME ANTIOXIDANTS ASSOCIATED WITH TOXOPLASMOSIS IN WOMEN WITH GESTATIONAL DIABETES

Jassim Enad Mahmoud¹, Dr. Omaima Ibraheem Mohamoud²
¹,² University of Tikrit / College of Veterinary Medicine

1. INTRODUCTION

Toxoplasmosis is a common parasitic disease caused by an obligatory intracellular protozoan parasite, Toxoplasma gondii. Many mammals and birds act as intermediate hosts while cats are the permanent hosts of the parasite (Saeed and Al-Aubaidi, 2020). Toxoplasmosis is one of the most common parasitic diseases affecting about one third of the world's population (Robert-Gangneux et al., 2017). In addition to being a serious and life-threatening disease, especially in pregnant women and people with immunodeficiency (Opsteegh et al., 2019). Infection generally occurs through consumption of undercooked meat containing tissue cysts or by water and food contaminated with parasite egg cysts present in cat feces (Jafari et al., 2012). The infection is transmitted directly from the mother to the fetus during the active phase (tachyzoite) through the placenta, rarely through blood transfusions or organ transplants (Vimercati et al., 2017).

Congenital toxoplasmosis occurs in pregnant women (Mahmood, 2016)). Transmission of the congenital Toxoplasma gondii parasite occurs mostly the first time during pregnancy (Al-Rawi, 2009). The severity of congenital toxoplasmosis is highest in the first and second trimesters of pregnancy, which usually leads to miscarriage or intrauterine death (Al-Sorji, 2005). As for acquired toxoplasmosis, it is not usually accompanied by the appearance of any symptoms, due to the resistance of the immune system in the body to it and preventing the emergence of these symptoms, as it appears similar to the symptoms of influenza in the early stages of infection (high body temperature, feeling of pain, fatigue and inflammation of the tonsils), but in the advanced stages it is Serious and life-threatening (it appears in the form of convulsions, pneumonia, enlargement of the liver and spleen, in addition to suffering from blurred vision due to severe inflammation of the retina) (Chaichan et al., 2017).

Diagnostic

Clinical signs of toxoplasmosis are non-specific and not sufficient to distinguish the disease, so the diagnosis of Toxoplasmosis infection in humans is done by several important methods, including biological, serological, histological and molecular methods (Robert-Gangneux and Dardé, 2012).

Direct Microscopical Examination

Diagnosis of proliferative phase in fluid samples such as blood, cerebrospinal fluid, bronchoalveolar lavage, milk, eye secretions, saliva, urine or swab made from biopsy or autopsy of skeletal muscle, lung, brain and eye to look for the presence of cysts in tissues (Piekarski, 2012). Rapidly proliferating crescent, circular or elliptical, cysts in tissues are usually spherical and lack a septum (Khalili et al., 2018).

Biopsies may show the rapidly proliferating phase or cysts stained with hematoxylin and eosin used in routine histopathological preparation in addition to Romanovsky's stains, e.g, Geimsa and Wright's, which are used to diagnose toxoplasmosis well (Liu et al., 2015).

Serological Tests

The most common serological tests for detecting the presence of IgG and IgM antibodies are the Sabin-Feldman stain test, the indirect fluorescent antibody (IFA) test, agglutination tests, or ELISA (Ayi et al., 2009).

Sabin-Feldman stain
In 1948, Sabin and Feldman developed a serological test called the dye test to make the serological diagnosis of toxoplasmosis, an infection caused by an intracellular parasite that affects all cells containing a nucleus in the human body. This test has radically changed our view of this mysterious clinical disease, which was previously associated with only a few cases of congenital and ocular toxoplasmosis (Pangalos et al., 1956).

Inderet Fluorescent Antibody test

This test was used for the first time in 1957 by the researcher Goldman to detect the antibodies specific to the Toxoplasma parasite. It was widely used, but it requires a fluorescent microscope, as the basis of its work depends on the principle of antigens linking with the antibodies present in the patient's serum, where it can detect antibodies IgM type This indicates acute infection. It is characterized by its high sensitivity and ease, and it gives positive results in very advanced stages (Trotta et al., 2016).

Indirect hemagglutination test (IHA)

The principle of this test is that red blood cells bound and sensitized to Toxoplasma antigen dissolved in the solution can cause hemagglutination when the parasite is present in the patient's serum as a positive test result. However, the detectability of IgG antibodies lags behind the dye test, so this test is likely to miss acute and congenital infections (Liu et al., 2015).

Latex Agglutination Test

In this test, a soluble antigen is coated on the latex particles, and agglutination is observed when positive patient serum containing antibodies is added. Latex agglutination is fast and easy to perform for the detection of IgG anti-Toxoplasma gondii. Latex agglutination sensitivity is 86-94% and specificity is 100% in humans. Thus, agglutination is often used as a screening tool in epidemiological surveys due to the simplicity of performance, but a positive result requires further investigation using other serological tests (Liu et al., 2015).

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is an important technique used primarily for the detection and detection of Toxoplasma IgG and IgM through sequential steps performed on patient serum. IgM indicates an acute infection, but it is now known that IgM may persist up to a year after infection (Iddawela et al., 2015).

Currently, pregnant women are tested in the first weeks of pregnancy for the presence of IgG and IgM antibodies to Toxoplasma gondii. The presence of Toxoplasma IgG at a constant level without IgM is due to a previous infection that has cleared. These women are considered immune to infection with toxoplasmosis and there is no need for follow-up treatment. In pregnant women, a routine monthly examination should be performed to diagnose and treat early acute infection in order to prevent transmission of the parasite across the placenta to the fetus. This highlights the importance of an accurate and reliable test to detect specific IgG, given that a false positive result can lead to a misdiagnosis of previous infection, discontinuation of surveillance and preventive measures in a pregnant woman. Therefore, other, more accurate methods must be found in the detection of toxoplasmosis, such as the polymerase chain reaction (PCR) test (Simon et al., 2020).

Study Aims

1- Diagnosis of Toxoplasmosis infection in pregnant women using the agglutination test as a primary test and comparing the results with the (ELISA) test.

II. MATERIALS AND WORKING METHODS

Samples of study

The study was conducted on 200 blood serum samples, 150 pregnant women with diabetes, 50 blood serum samples from non-diabetic women as control samples. Samples were collected in Balad General Hospital, Yarmouk General Hospital, Al Alawia Maternity Hospital, as well as in private laboratories for the period from October 2020 to January 2021. After conducting a rapid examination and ELISA examination of the patients, the samples were divided into 4 groups to study the levels of antioxidants, where the first group included uninfected women (control), the second group included women with diabetes only, and the third group included women infected with Toxoplasma parasite, and the last group included women She has diabetes and the Toxoplasma parasite.
Blood samples collection and serum preparation
Blood samples were collected in the current study by withdrawing (10 ml) of venous blood through a single-use syringe, and then divided into two parts: 3 ml was placed in a test tube containing an anticoagulant substance EDTA and (7 ml) was placed in plastic tubes with tight lids. And free of anticoagulant, the tubes were left at room temperature (25 degrees Celsius) until coagulation was completed and were placed in a centrifuge for 10 minutes at a speed of 3000 rpm, then the serum was withdrawn by means of a micropipette and placed in clean, sterile tubes and kept in a frozen state At a temperature of (-20ºC) until carrying out the examinations under study.

Toxoplasmosis Rapid Examination:
A- Principle of the Method:
The LINEAR Toxo IgG / IgM cassette is designed to be used as a screening test for Toxoplasma gondii infection and is a chromatographic immunoassay. The test strip consists of: 1) a burgundy conjugate containing T. gondii antigens conjugated with gold particles and the conjugated and conjugated rabbit IgG immunoglobulin With gold particles, 2) a nitrocellulose membrane strip containing two test strips (M and G) and a control strip (C). The M strip is pre-coated with mono-anti-human IgM for the detection of toxoplasma immune antigens (IgM anti-T. gondii), the G strip is pre-coated with reagents for the detection of anti-T.gondii IgG antibody, and the C strip does not contain Antigens (Lykins et al., 2018).

b- Method of Working:
Leave the test device, samples and control to settle at room temperature (15-30°C) prior to examination.

1- Remove the cap from the test device and use it as soon as possible on a clean, flat surface.

2- Ensure that the test device is named with the sample ID number where the blood sample is used.

3- Place a drop of whole blood (approximately 40-50 μl) into the sample hole.

4- Then add 1 drop (approximately 35-50 μl) of the diluted sample immediately.

5- Set the timer and read the result within 15 minutes.

2.2.3 IgM/IgG Toxoplasma ELISA Test
A- The principle of the method:
The examination was conducted according to the method of the American company Biocheck, the manufacturer of the used kit. The ELISA assay is based on the correlation between the Toxoplasma gondii Ag5 antigens that cover the surface of the pits for the performance of the examination and the IgM and IgG antibodies present in the diluted serum to be fermented when they are present in it. (Muhsin et al., 2013).

B - Method of work: The method of ELISA examination works as in the following table:
1- The serum samples and the test kit were placed to reach room temperature. Should the examination begin?

2- Both serum samples, positive and safe control were diluted in a ratio of 1:40, i.e. by adding 200 μl of dilution solution to 5 μl of serum to dilute the serum and 200 μl of dilution solution to 5 μl of dilution solution to the negative control to dilute the control samples.

3- The washing solution was prepared by adding 950 μl of distilled water to (50 μl of concentrated working solution (20x).

4- 10 μl of pre-diluted samples and positive and safe control serum were added to the micro wells for the examination disease, taking into account the absence of air bubbles.

5- Hams were covered with a cover to prevent evaporation, and then incubated for 30 minutes at 37 degrees.
6- The pits were washed after the expiry of the induction period with a Microtiter plate washer and using the pre-prepared washing solution for five consecutive times.

7- 100 μl of conjugated enzyme reagent was added and the plate was moved to the concave surface for 10 seconds and then the holes were washed again and for five consecutive times as well.

8- Add 100 μl of TMB reagent to each hole and stir the plate for 10 seconds

9- The pits were covered with the adhesive barrier and incubated at 37 m for 15 minutes

10 - The solution to terminate the reaction IN HCL was added after the incubation period with stirring for 30 seconds, and it was noticed that the color turned blue to yellow completely in the positive samples.

11- I read the samples using the ELISA reading device at a wavelength of 450 nm.

III. RESULTS AND DISCUSSION

Toxoplasmosis Infection Rates by Type of Examination

The results of the immunodiagnosis of 200 serum samples of women infected with Toxoplasmosis in the governorates of Baghdad and Salah al-Din using the Rapid test showed that 49 samples were positive and a percentage of (24.5%), and 151 samples were negative and a percentage of (75.5%) for the IgG-test. Rapid test. It was also found that the results of the IgM-Rapid test were only two positive samples with a percentage of (1%).

The diagnosis was confirmed using the ELISA IgG assay, there were 71 positive samples with a percentage of (35.5%) and 129 negative samples with a percentage of (64.5%), and using the ELISA-IgM assay, where there were 2 positive samples with a percentage of (1%) and 198 negative samples, with a percentage of (99%). Figure (1 and 2) shows the numbers of tested and infected samples and the percentages of infection according to the type of test. The results of the current study are in agreement with the results of a clinical study conducted to investigate the infection of toxoplasmosis in patients with diabetes in the Kingdom of Saudi Arabia, where the results indicated an increase in the level of IgG immune antigen in the blood of patients by a large percentage compared to the proportion of IgM (Ismail, 2018). The results of the ELISA test showed a significant increase in the proportion of IgG antigen compared to IgM antigen in pregnant women with gestational diabetes (Shakiba et al., 2020).

![Figure 1: Results of toxoplasmosis in women under study using an IgG ELISA test.](image)

Figure (1) shows a significant increase in the level of absorbance of IgG antibodies for the positive group of pathogens, which reached a rate of (1.558), and there was a significant decrease in the rate of absorbance of antibodies to the negative group of pathogens, which amounted to (0.439). The statistical analysis indicated that there were significant differences, P-Value = 0.0005.

A study conducted by (Gras et al., 2004), IgG antibodies appear after about one to two weeks after infection and reach a peak in (8-6) weeks and then decrease gradually over a period of (1-2) years, and the low titer may persist for a long time. Life in some infected women has a very high titer that lasts for several years and therefore it
plays an important role in reducing and controlling toxoplasmosis infection and preventing the spread of the parasite due to its long survival.

Figure (2) shows a significant increase in the level of absorbance of IgM antibodies for the positive group of pathogens, which reached a rate of (1.513), and a significant decrease in the absorbance of antibodies to the negative group of pathogens, which amounted to (0.16). The statistical analysis indicated that there were significant differences, P-Value = (0.049).

The results of the current study agree with the results of an immunological study of Toxoplasma parasite in pregnant women, which proved that the levels of IgG antigen rise in pregnant and non-pregnant women by a very large percentage compared to the IgM antigen because the IgG antigen remains for longer periods in the bodies of women infected with the parasite. Parasite infection in pregnant women causes some miscarriages and miscarriages may be repeated several times, but the highest rates of miscarriages remain in the first pregnancy (Shaker et al., 2018).

The results of another study showing the extent of infection in Toxoplasma parasite in diabetic patients in Baghdad governorate, when measuring the uptake of IgM and IgG antibodies, indicate a significant increase in the level of IgG in diabetic patients compared to healthy women, while the level of IgM did not change significantly. This is due to the fact that women at The infection with acute toxoplasmosis did not perform the examination to detect the parasite in the hospital laboratories within 14 days, which caused them to form IgG, and chronic infection was recorded (Hilal and Hamad, 2019).

The results of Figure (1) and (2) indicate a rise in the level of absorption of IgM and IgG antibodies. This can be explained by the fact that the levels of specific antibodies against Toxoplasma parasite for women who have been exposed to infection still show a significant increase and at low concentrations after very long periods compared to women who have not been infected. In the disease, as the rise of the IgM antigen two weeks after infection is an indicator of the appearance of the infection in the first week, reaching its highest rise in the second and third weeks, then it begins to decline and shows a clear rise in the level of IgG antigen in this period gradually and for several months, then decreases as it remains for long periods of time. It reaches a lifetime (Fricker-Hidalgo et al., 2020; Villard et al., 2016).

The groups most at risk of contracting this disease are pregnant women, because of the parasite's ability to cross the placental barrier and infect the fetus before it acquires immunity. Other at-risk groups are immunocompromised patients who are susceptible to infection with the Toxoplasma parasite (Weiss and Dubey, 2008).
The immune system is affected in diabetics, and this makes them susceptible to infection with Toxoplasma parasite due to the weakening of the immune system, especially beta cells. Gestational diabetes patients are more susceptible to infection with Toxoplasma gondii, as it was noted that the parasite infection rate was significantly higher when the parasite prevalence was studied among groups of diabetic patients of other types (Li et al., 2018).

The results of the current study agree with many studies conducted to investigate the Toxoplasma parasite, which showed an increase in the proportion of IgG immune antigen in diabetic patients infected with the parasite more than the proportion of the immune antigen, which showed no significant increase (Hadi and Alomashi, 2018; Sakir et al., 2016; Kanková et al., 2015). The high percentage of IgG immune antigen in gestational diabetes patients indicates the presence of a chronic infection, as studies have shown that the percentage of chronic infection is greater than the percentage of acute toxoplasmosis infection for diabetic patients (Ali Saeed and Al-Aubaidi, 2018; Molan and Ismail, 2017).

REFERENCES

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